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A Comparison of Two *Vannella* Species

ROBERT F. MOTE

Abstract. Two species of *Vannella* from a freshwater pond were cultured in distilled water, alfalfa, rice medium and described. Both species had a cyst form, radiate form, and trophic form. One, *Vannella multimorpha* sp. nov., was distinct in that large uni- and bi-nucleated *Vannella* were formed during the life cycle. From the large trophic *Vannella multimorpha* small amoebae buds were developed. Illustrations of the life cycle and microphotographs of this new species are represented.

The evolution of the Sarcodina has spanned millions of years and their complexity is only beginning to be understood. The genus *Vannella* represents the trophic form of a polymorphic organism that also includes a radiate stage.

Ehrenberg (1838) was the first to describe a radiate amoeba and to give it the name *Amoeba radiosa*. Since that time several generic names have been used for various radiate forms. Claparède and Lachmann (1858) suggested the genus *Podostoma*. The genus *Dactylospherium* was suggested by Hertwig and Lesser (1874). Vejdovsky (1881) proposed the genus *Astramoeba*, and Bovee (1951) proposed the genus *Flagellipodium*. Dujardin (1841), Leidy (1879), Penard (1902), Cash and Hopkinson (1905), Schaeffer (1926) and others have commented on or described radiate forms under one of the above generic names.

Since Ehrenberg's description in 1838 the radiate forms have caused concern. Bovee (1951) states the "radiate forms of the free-living amoebas are only patterns assumed when the organisms are least active in daily patterns of movement or upon being disturbed. There is, therefore, no such thing as a valid *Amoeba radiosa*, nor any need resultantly for any genus to include the radiate forms of naked amoeba which in active locomotion progress by amoeboid movement." However, one is reluctant to dismiss *Amoeba radiosa* as a valid species. Several species of radiate amoebae may have a size range comparable to that reported by Ehrenberg. Various species of amoeba may develop a radiate form when subjected to stimulation. Bovee (1965) proposed the genus *Vannella*, and Bovee and Jahn (1966) presented a taxonomic classification for the small amoeba species. For the genus *Vannella* a complete knowledge of the life cycle of the organism is necessary. This study is concerned with culture methods and the life cycle of two *Vannella* species.

MATERIALS AND METHODS

The radiate organisms were collected during May and June, 1966, from the freshwater pond located at 34th Street and Whitmer Parkway, Des Moines, Iowa. A small amount of ooze was obtained from the east end of the pond where emergent vegetation is present during

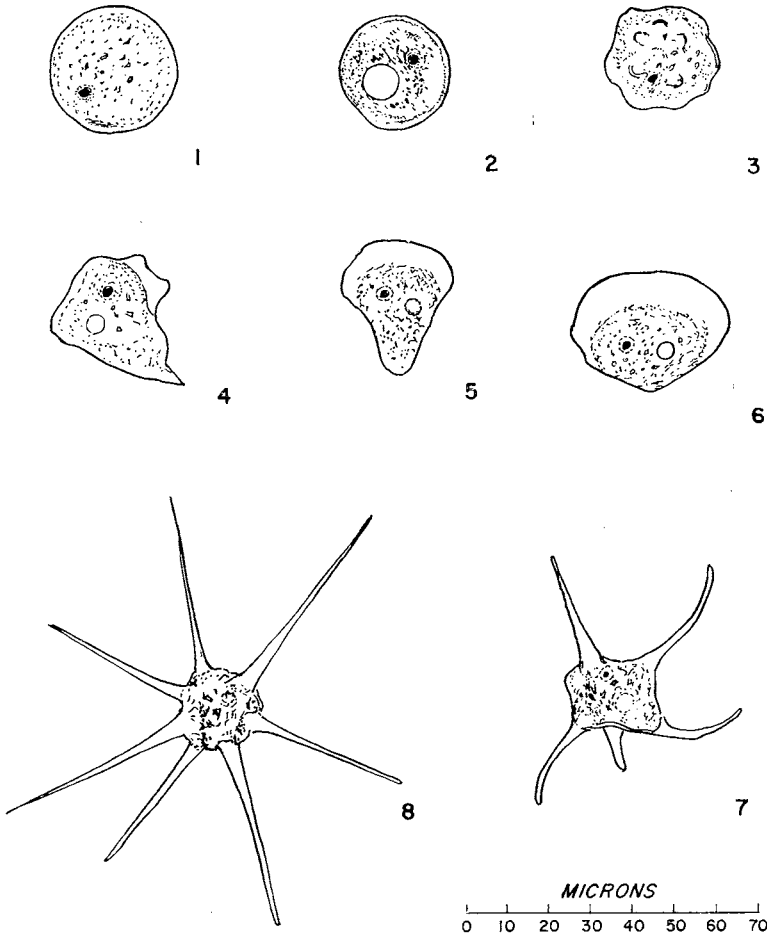
the summer. When samples were examined, occasional radiate forms were placed in a medium containing distilled water, alfalfa, and rice. During the winter of 1966 the two radiate species were isolated and individually placed in clones. The distilled water content was reduced from that in the medium previously described by Mote (1967). Seventeen cc. of distilled water was placed in flat bottom biological shell glass test tubes (25 x 95 mm.) which had been previously washed, air-dried, and cotton stoppered. They were autoclaved at 15 pounds pressure for 20 minutes. Two 25-30 mm. alfalfa stems were boiled for 15 minutes and then added to the test tubes. Rice particles were boiled for five minutes and 0.75 of a particle was added to each test tube. After cooling, bacteria were added (Mote, 1966). After 72 hours the amoeba were inoculated into the cultures. For each of the two species of *Vannella*, three test tubes a week were prepared for 10 weeks during the summer of 1967. Micropipettes were autoclaved after single usage. Microscopic observation and description was from living culture samples and prepared microscope slides. Nissenbaum's (1953) fixative with Heidenhain's (1892) Iron Hematoxylin was the method for prepared slides. Examination was by a Bausch and Lomb research microscope. A calibrated ocular micrometer disk was used to determine the locomotion rate.

RESULTS

Vannella sp. A. (Figures 1-6, 16-19)

The cysts: Round temporary cysts were observed in the laboratory cultures. They readily gave way to active amoebae during microscopic observation (Figure 1). The cyst membrane is simple with the appearance of a highly refractile layer, 1 μ in thickness, just below the membrane surface. The cysts are filled with granulated protoplasm and have a modal diameter of 30 μ . The vesicular nucleus is easily visible in life. Figure 2 shows the indentation commonly seen as the temporary cyst becomes active. At this time the refraction layer is not as prominent or may be completely absent. The membrane assumes a wrinkled form that transforms into a many papillated surface. Rotation of the cystlike form becomes evident and lateral extensions from the papillae may indicate pseudopodium formation. The observed patterns in the life cycle of this particular *Vannella* species are:

1. The transition of the indented cyst form (Figure 2) to the transitional stage (Figure 4) and then immediately into the trophic non-floating form (Figures 5, 6).
2. The transition of the wrinkled papillate form (Figure 3) to the radiosa form (Figures 7, 8).
3. Withdrawal or transformation of the wrinkled papillae of the cystlike form with the formation of clear ectoplasm at one side leading to the trophic form (Figures 3-6). The ectoplasm develops at the an-



Vannella sp. A. Figure 1. The temporary cystlike form. Figure 2. The indented cystlike form. Figure 3. A papillated form. Figure 4. A transitional form. Figure 5. A slow moving trophic form. Figure 6. A rapid moving trophic form. Figure 7. The beginning of the radiate form. Figure 8. Fully formed radiate form.

terior part of the trophic form; however, in the active condition active metaboly may sometimes be observed before a definite direction is taken by the organism.

4. From a partly formed radiate form, one of the pseudopods withdraws and the clear ectoplasm expands into the motile form.

Radiate form. From the wrinkled papillae a rounded granular papilla extends to form the pseudopodium. In this species the pseudopods are not the same width at the base, and a variety of pseudopodial

shapes from curved to straight may be seen (Figure 7, 8). They may sway actively. The number of pseudopodia varies from 4-11. In the floating condition the pseudopodia are completely radiate and they do not sway back and forth. When fully extended the pseudopods are rigid thin rays extending from the central protoplasmic mass (Figure 8).

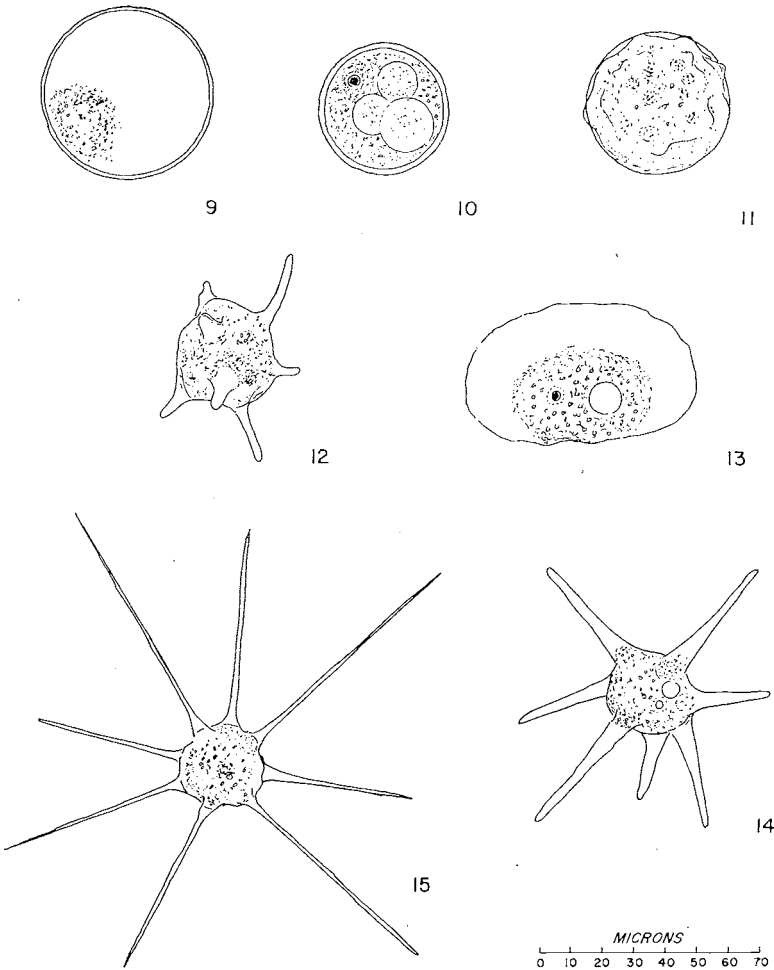
Trophic form. A clear ectoplasmic zone (from or between) the papillae can also give rise to the trophic organism. The ectoplasmic flow results in a flattened compressed form where the length may be greater than the width. A triangular form with a clear ectoplasmic cap indicates a slow moving organism (Figure 5). The organism may then approach a fan shape. This species has a fast-moving speed of 55μ a minute. The modal size for the trophic amoeba is $30 \times 37.5 \mu$ (Figure 6). The nucleus is vesicular with a round endosome; the amoebae are usually uninucleate. The endosome has a diameter of 2.25μ with a total nuclear size of 4.5μ . The nucleus is highly refractive and delicate green in color. A cross-sectional ratio of one-half clear ectoplasm to one-half granular endoplasm is common in the trophic form. With fast-moving fan-shaped forms the ratio of clear ectoplasm may be greater. One contractile vacuole, $6-10 \mu$ in diameter, is formed by coalescence of smaller vesicles which vary in size. The granular ectoplasm of the trophic form has particles up to 1μ , and with iron hematoxylin the clear ectoplasm shows a very fine granulation except at the extreme anterior periphery.

Division. Division takes place in the motile stage by a constriction that separates the daughter organisms (Figure 19). Lateral extensions of the two halves are attached by a thread of protoplasm until the individuals are separated. Opposite to the cleavage plane the clear anterior ectoplasm can be seen. Multiple fission has been observed (Figure 18), and in this species some organisms are binucleate in the motile form (Figure 17).

Vannella multimorpha sp. nov. (Figures 9-15, 20-27)

An unusual characteristic of this freshwater *Vannella* was the formation of small exogenous amoeba buds that appeared in laboratory cultures.

The cysts. The cysts for this species ranged from $45-60 \mu$ in diameter with a few reaching 70μ . These varieties of *V. multimorpha* cysts were seen. In older cultures the protoplasm was concentrated toward one side of the cyst. Development of this large cyst was not observed into a radiate or trophic form. A granular cyst, $45-52 \mu$, during microscopic observation gradually gave way to form a radiate or trophic amoeba. The cyst had a refractive layer just below the cyst membrane that measured 1.25μ in thickness, and vesicular nucleus with a large prominent endosome (Figure 10). The modal over-all nuclear diam-



V. multimorpha sp. nov. Figure 9. A large inactive cyst. Figure 10. A typical temporary cyst that can give rise to a radiate or trophic *V. multimorpha*. Figure 11. Occasional wrinkled cystlike form observed. Figure 12. A transitional stage with beginning pseudopod formation. Figure 13. A characteristic trophic form in rapid locomotion. Figure 14. An incomplete radiate form. Figure 15. An extended radiate form of *V. multimorpha*.

eter was $7.7\ \mu$ and the endosome $4.60\ \mu$. Within some granular cysts one to three vacuolar rings were present while others had none. The vacuolar rings may be quite large (Figure 10). Fine to coarse granules, up to $1.5\ \mu$ in size, are present in the protoplasm of the granular cysts. Occasionally wrinkled cysts were observed (Figure 11).

Of the three cyst varieties the granular temporary form differenti-

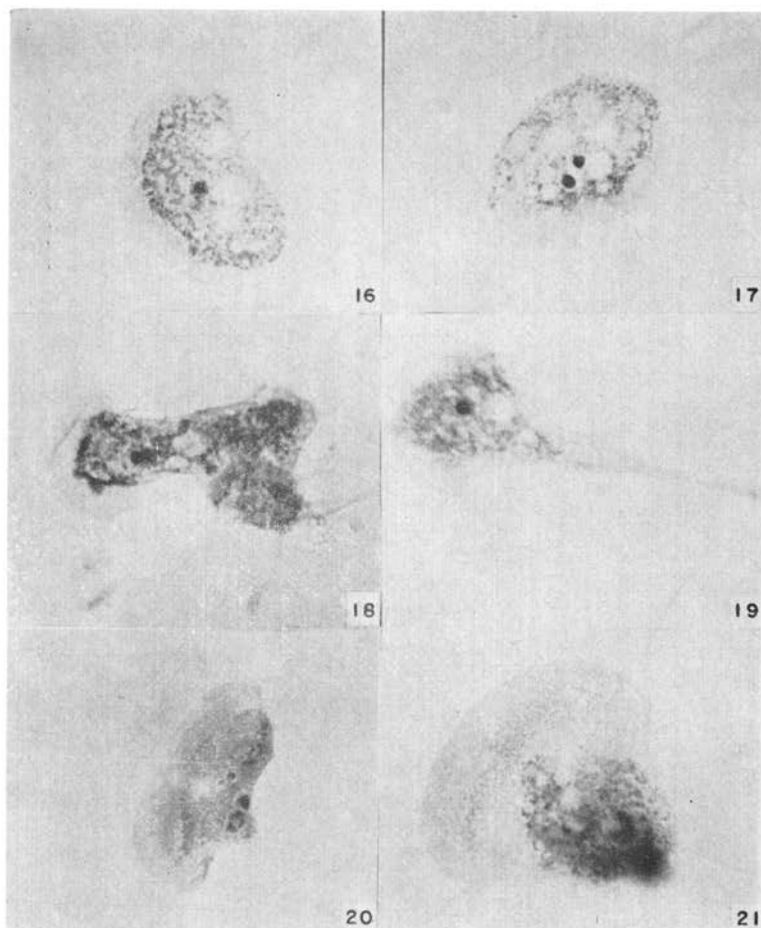


Figure 16. A microphotograph showing the single nucleus of the active trophic form. *Vannella* sp. A. x907. Figure 17. A binucleated trophic form in active locomotion. *Vannella* sp. A. x968. Figure 18. Multiple fission observed in *Vannella* sp. A. x478. Figure 19. The thread of *Vannella* indicating incomplete separation. x904. Figure 20. *V. multimorpha* approaching the rapid locomotion form from the slow moving form. x248. Figure 21. A slow locomotion form of *V. multimorpha*. x598.

ated consistently into a transitional stage leading to the formation of either a radiate or trophic *V. multimorpha*.

Radiate form. Small blunt pseudopods were extended from the transitional form (Figure 12). As many as 14 pseudopodia have been observed extending from the transitional form. As some were extended others were being reabsorbed. The pseudopodal width next to the central body of the radiate form was 11 μ . The pseudopodia were wide

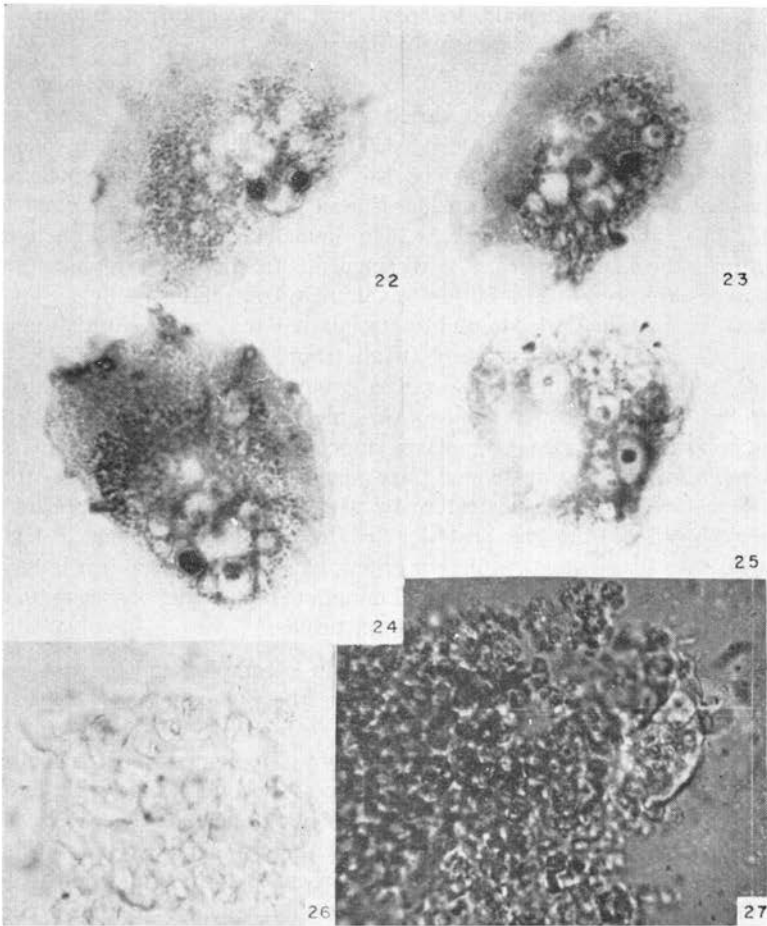


Figure 22. A binucleated *V. multimorpha*. x512. Figure 23. A large *V. multimorpha* with pronounced dark staining material in the vacuoles. x445. Figure 24. The characteristics of a large *V. multimorpha*. x509. Figure 25. *V. multimorpha* showing the contractile vacuole at systole and prominent laden vacuoles. x374. Figure 26. A living microphotograph of small amoeba buds adhering to a large *V. multimorpha*. x713. Figure 27. The clear ectoplasm of a large *V. multimorpha* with small pigmented amoeba buds.

with rounded blunt tips; however, the pseudopods became more slender as they extended. The total diameter of the extended radiate form may reach $175\ \mu$. The pseudopodia do not contain endoplasmic granules. Prepared slides showed faint staining granules of the motile ectoplasm that may have the same morphological characteristics as those appearing in the base of the pseudopods of the radiate form. In this organism a reduction of the pseudopods takes place from the transitional form to the fully extended radiate form. When fully

extended, the pseudopods are rigid and a reduction in number of pseudopods to nine is common for this species.

Trophic form. The trophic form of *V. multimorpha* was large for *Vannella*. The motile forms varied from 45-60 to 75-120 μ , and very large binucleated forms reached 125 μ in width. Many fan-shaped organisms with a single nucleus had a width of 80 μ . Binucleated amoebae are common. The nucleus is vesicular with an over-all diameter of 7.68 μ , and an endosome 5.59 μ in diameter (Figure 20). In living cultures the nucleus is easy to distinguish. In the large trophic forms vacuoles are present in addition to the main contractile vacuole. These vacuoles contain dark staining material that may not be nutritive as they do not occur consistently in all trophic forms (Figure 23). At systole the contractile vacuole ranged from 7.5 μ to 11 μ . The rate of locomotion was 80 μ per minute. Granules up to 1 μ are found in the endoplasm. The clear ectoplasm appears more abundant than the endoplasm in the fan-shaped fast-moving form. The radiate form attaches itself to the substratum by a clear ectoplasm and appears to be reabsorbed from the posterior half of the trophic form. Pseudopodal rays may extend while the anterior half of the organism is being formed; however, the complete elimination of pseudopods soon takes place. *V. multimorpha* does not form uroids.

Division. The division of this organism is complex and has not been completely determined. Binary fission is the usual method. However, in older cultures small amoeba buds were observed. The amoeba buds, in structure, are similar to the exogenous buds described and illustrated by Hogue (1914). Although larger, the buds range from a few microns to 15 μ and lack a distinct vesicular nucleus. The buds contain small particles which stain dark with iron hematoxylin. Failure to find many developing small amoeba in cultures, in proportion to the number of small amoeba buds, may indicate a lack of essential chromatin. Several repeated washings in distilled water and culturing of a single *V. multimorpha* organism continued to indicate amoeba bud formation in older cultures. Taylor (1924) suggested a form of sporulation for *Amoeba proteus*. Small amoebae that had the morphology of *V. multimorpha* were observed, but they were not numerous.

DISCUSSION

Schaeffer (1926) described *Flabellula citata* as a marine form that can be experimentally adapted to fresh water. *F. citata* was unusual when placed in distilled water according to Schaeffer; a large number of small amoeba were found in the culture medium, but most of these did not survive. *F. citata* produced numerous contractile vacuoles, but did not form cysts. *F. citata* did include a rayed stage, but in the trophic form uroids were always present.

Hogue (1914, 1921) presented descriptions of two new species,

Valkampfia patuxent and *Valkampfia calkensi*. Both formed cysts that were dissimilar. *V. multimorpha* described above formed large cysts and was uninucleate or binucleate in the trophic form. *V. patuxent* and *V. calkensi* was multinucleated in the trophic form; however, no rayed form is described for either. Miss Hogue states that *V. calkensi* and *V. patuxent* experimentally produced small amoebas by exogenous and endogenous division. Schaeffer (1926) concludes that "Miss Hogue ('14, '21) has described two species of amoebas; *Valkampfia calkensi* and *V. patuxent*, both of which resemble *Flabellula citata* in the locomotive stage and should therefore be included in the genus *Flabellula*. There is a possibility that the latter species (*F. citata*) is a synonym of one or other of Miss Hogue's amoebas." Bovee (1965) has suggested that *V. calkensi* and *V. patuxent* be placed in *Flabellula*.

The freshwater organism described here had definite morphological characteristics: no uroids, a cyst form, a radiate form, a very active trophic form with a single nucleus, large binucleate trophic forms, and small amoeba buds formed in the culture medium. Because of these characteristics I propose the name *V. multimorpha* nov. sp.

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